JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2006, p. 4292–4294 Vol. 44, No. 11
0095-1137/06/$08.00+0 doi:10.1128/JCM.01577-06

Supplemental Testing Is Still Required in Australia for Samples Positive for *Neisseria gonorrhoeae* by Nucleic Acid Detection Tests

The introduction and widespread application of nucleic acid detection tests (NADT) for *Neisseria gonorrhoeae* (i.e., gonococci [GC]) in Australia has enhanced diagnostic capacity and public health control of gonococcal disease. However, the well-recognized problems with the use and interpretation of GC NADT in the difficult and widely varying conditions in Australia led to a formal request for a specially convened expert reference group (ERG) to develop guidelines relevant to these different situations (9). We therefore noted with some concern conclusions by Lowe et al. (4) suggesting that supplemental testing for diagnosis of gonorrhea using GC NADT is not required in Australia when initial testing is by the Gen-Probe APTIMA Combo 2 (ACT) assay. This assertion contradicts the recommendations contained in the national consensus guidelines that were based on a review of the published literature and the extensive experience obtained over many years in the diverse test conditions experienced here. The ERG guidelines are consistent with another recent review of GC NADT testing in Australia (11) and the current National Pathology Accreditation Advisory Council of Australia (NPAAC) guidelines (7). The latter supersedes advice contained in the earlier NPAAC (6) guidelines on which Lowe et al. (4) relied.

There were a number of important factors that were subject to formal consideration by the Australian ERG seemingly ignored by Lowe et al. (4). First, this ERG (9) pointed to problems with GC NADT sensitivity that may result from the capacity of *Neisseria gonorrhoeae* to repeatedly recombine/alter its genome through acquisition or loss of genetic sequences. These alterations result in gain or loss of important characteristics not only for NADT-based diagnosis but also for culture-based analyses (3, 8). These subpopulations may vary quite substantially as a proportion of the total gonococcal population in different patient subgroups and over time and place (3, 5). The test numbers mentioned by Lowe et al. (4) suggest that their study was limited to a sample obtained over approximately 1 month only, which is insufficient to properly assess any GC NADT.

Second, the Australian guidelines (9) also emphasized that cross-reactions with non-gonococcal *Neisseria* strains remains a significant limitation of GC NADT and that specificity may also vary depending on the patient population. Lowe et al. (4) mention that the AC2 "does not cross-react with other *Neisseria* spp." To our knowledge, this has not been extensively investigated in Australia or elsewhere. Hence, the high clinical specificity of the AC2 observed by Lowe et al. (4) may not necessarily reflect the performance of the AC2 in other patient populations, either within or outside Australia. Additionally, the numbers of assays positive for GC were very low ($n = 39$), not all four GC NADT were used in all instances introducing a bias into the "resolved" data, and the population (not specifically defined) was seemingly a low-prevalence group, further compromising the assessment.

Third, experience in Australia has shown us that commercial GC NADT satisfying existing FDA standards and the earlier NPAAC guidelines (6) but using limited prerelease evaluation have had to be subsequently withdrawn from use in Australia (9). Some "in-house" assays (5) were similarly withdrawn after more extensive and complete "in-use" appraisals revealed substantial deficiencies in performance. The relatively small number of samples examined by Lowe et al. (4) would not reliably detect these problems.

Also in an Australian context, Lowe et al. relied on a *cppB*-based supplemental assay as their final "confirmatory" test (4), despite the fact that the use of this assay as both a screening and supplemental assay has been associated with false-negative and false-positive results both in Australia and elsewhere (1, 3, 9, 10).

It is important that GC NADT is subjected to rigorous, extensive, and continuing evaluation before being endorsed as being suitable for use as a single definitive diagnostic test. It is our view that Lowe et al. (4) failed to satisfy the principles and details required for proper appraisal of this GC NADT, particularly in an Australian context. Data such as those presented may be relevant to some situations and do contribute to the wider appraisal that we recommend. However, an incomplete evaluation performed at one geographic location at one time point on small numbers of positive cases is essentially meaningless if it is to be used, as implied by Lowe et al., as a basis for universal test validation for a particular GC NADT.

In the opinion of both the Australian ERG (9) and other national bodies (7), a modification of the Centers for Disease Prevention and Control approach (2) that includes supplemental testing provides a reasonable and pragmatic basis for reliable reporting of results of GC NADT in Australian conditions. It was also the considered opinion of the ERG (9) that the available local data on AC2 assays were to date incomplete and that total reliance on manufacturers' data to remedy this deficiency may be misplaced. The additional information supplied by Lowe et al. (4) does not alter these views.

The Public Health Laboratory Network of Australia and the National Neisseria Network of Australia endorsed the opinions expressed above.

REFERENCES

1. **Bruisten, S. M., G. T. Noordhoek, A. J. C. van den Brule, B. Duim, C. H. E. Boel, K. El-Faouzi, R. du Maine, S. Mulder, D. Luijt, and J. Schirm.** 2004. Multicenter validation of the *cppB* gene as a PCR target for detection of *Neisseria gonorrhoeae*. J. Clin. Microbiol. **42:**4332–4334.
2. **Johnson, R. E., W. J. Newhall, J. R. Papp, J. S. Knapp, C. M. Black, T. L. Gift, R. Steece, L. E. Markowitz, O. J. Devine, C. M. Walsh, S. Wang, D. C. Gunter, K. L. Irwin, S. DeLisle, and S. M. Berman.** 2002. Screening tests to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections 2002. Morb. Mortal. Wkly. Rep. **51**(RR-15)**:**1–48.
3. **Limnios, E. A., N. L. Nguyen, S. Ray, C. J. McIver, and J. W. Tapsall.** 2006. Dynamics of appearance and expansion of a prolyliminopeptidase-negative subtype among *Neisseria gonorrhoeae* isolates collected in Sydney, Australia, from 2002 to 2005. J. Clin. Microbiol. **44:**1400–1404.
4. **Lowe, P., P. O'Loughlin, K. Evans, M. White, P. B. Bartley, and R. Vohra.** 2006. Comparison of the Gen-Probe APTIMA Combo 2 assay to the AMPLICOR CT/NG assay for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in urine samples from Australian men and women. J. Clin. Microbiol. **44:**2619–2621.
5. **Lum, G., K. Freeman, N. L. Nguyen, E. A. Limnios, S. N. Tabrizi, I. Carter, I. Chambers, D. M. Whiley, T. P. Sloots, S. M. Garland, and J. W. Tapsall.** 2005. A cluster of culture positive gonococcal infections but with false-negative *cppB*-gene-based PCR. Sex. Transm. Infect. **81:**400–402.
6. **National Pathology Accreditation Advisory Council.** 2000. Laboratory accreditation standards and guidelines for nucleic acid detection. Australian Government Department of Health and Ageing, Canberra, Australia.
7. **National Pathology Accreditation Advisory Council.** 2006. Laboratory accreditation standards and guidelines for nucleic acid detection and analysis.

Australian Government Department of Health and Ageing, Canberra, Australia.
8. **Palmer, H. M., H. Mallinson, R. L. Wood, and A. J. Herring.** 2003. Evaluation of the specificities of five DNA amplification methods for the detection of *Neisseria gonorrhoeae*. J. Clin. Microbiol. **41:**835–837.
9. **Smith, D. W., J. W. Tapsall, and G. Lum.** 2005. Guidelines for the use and interpretation of nucleic acid detection tests for *Neisseria gonorrhoeae* in Australia: a position paper on behalf of the Public Health Laboratory Network. Commun. Dis. Intell. **29:**358–365.
10. **Tapsall, J. W., E. A. Limnios, N.-L. Nguyen, I. Carter, G. Lum, K. Freeman, S. N. Tabrizi, S. M. Garland, D. M. Whiley, T. P. Sloots, and I. W. Chambers.** 2005. Cryptic-plasmid-free gonococci may contribute to failure of *cppB* gene-based assays to confirm results of BD ProbeTEC PCR for identification of *Neisseria gonorrhoeae*. J. Clin. Microbiol. **43:**2036–2037.
11. **Whiley, D. M., J. W. Tapsall, and T. Sloots.** 2006. Nucleic acid amplification testing for *Neisseria gonorrhoea:* an ongoing challenge. J. Mol. Diagn. **8:**3–15.

G. Lum
NT Government Pathology Services
Royal Darwin Hospital
Tiwi, Northern Territory, Australia

S. M. Garland
S. Tabrizi
Department of Microbiology and Infectious Diseases
Royal Women's Hospital
Melbourne, Victoria, Australia

G. Harnett
D. W. Smith
Division of Microbiology & Infectious Diseases
PathWest Laboratory Medicine WA
Nedlands, Western Australia

T. P. Sloots
D. M. Whiley
Queensland Paediatric Infectious Diseases Laboratory
Sir Albert Sakzewski Virus Research Centre
Royal Children's Hospital and Health Service District
Queensland, Australia

J. W. Tapsall*
Microbiology Department
South Eastern Area Laboratory Services
The Prince of Wales Hospital
Randwick, Sydney, Australia

*Phone: 61-293829079
Fax: 61-293984275
E-mail: tapsallj@sesahs.nsw.gov.au

Authors' Reply

We appreciate the comments provided by Lum et al. (referred to as "the authors" throughout this reply) regarding our publication. The authors raise points which we believe warrant reply.

The authors appropriately highlight the well-known difficulties encountered in the confirmation of positive screening with *Neisseria gonorrhoeae* nucleic acid amplification tests (GC NAAT) (14, 17). They highlight the *cppB* gene assay and three others developed or being evaluated by members of the expert reference group (ERG) (*porA* pseudogene, *opa*, and a cytosine DNA methyltransferase gene assay [1, 3, 14–16]). We support the authors comments referring to the problematic genetic variation associated with these and other potential targets and the impact on diagnostic testing. However, none of these genes are targets for the APTIMA Combo 2 (AC2) or APTIMA *Neisseria gonorrhoeae* (AGC) assays (Gen-Probe Incorporated, San Diego, CA).

The AC2 assay has been in the marketplace since 2001 and extensively tested on four continents with comparable results. We are unaware of published reports of either assay (AC2 or AGC) cross-reacting with non-gonococcal isolates as yet.

Peer-reviewed studies in the public domain since 2003 (2, 4–7, 11) have detailed the diagnostic performance of the AC2 assay, including in low-prevalence populations (7), and have also highlighted that both the AC2 and AGC assays target different regions of the 16S rRNA gene (5). Comparison with earlier work (8) clearly contradicts the authors' assertion (14) that the AGC assay does not demonstrate major divergences from "an earlier version of the same assay." Gen-Probe, in fact, did not have an amplified GC assay on the market before the AC2 assay. The AC2 assay was FDA cleared in August 2001 for the semiautomated systems, while the AGC was FDA cleared in March 2005 for the same systems (Gen-Probe Incorporated, San Diego, CA). At the time of our evaluation, we used the analyte-specific reagent version of the product.

Good laboratory practice requires that each laboratory evaluate the performance and appropriate use of any test for the population of patients to which it provides services. The aim of our study was to compare the AC2 assay to our then existing testing technology (Roche AMPLICOR CT/NG for detection of *Chlamydia trachomatis* and GC with confirmation of GC positives by *cppB* gene detection) in our referral population. Our criteria for the determination of "true positive" results were based on published contemporary Australian and American guidelines (9, 12), i.e., repeat testing of all positives using another set of primers directed at a different target sequence. The National Pathology Accreditation Advisory Council of Australia guidelines (13) were in draft form at the time of submission. The relevant sections quoted by the authors are identical to the guidelines published in 2000 (12).

We are unclear how the authors concluded that our study was conducted over a 1-month period with samples from one geographic location. Our sampling of specimens was conducted from April to September 2004. QML Pathology provides private referral outpatient and inpatient diagnostic services across the state of Queensland, Australia (population, approximately 4 million), comprising 22.5% of the Australian land mass. We perform over 75,000 tests for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* per annum. Our state-wide catchment area includes low- and high-prevalence populations, including indigenous and nonindigenous Australians. We therefore believe that the selection timeframe and the diversity of our population catchment area to be at least reasonable and certainly negates the points raised in this regard by the authors.

Additionally, the authors inaccurately quote the number of true positives with GC (45; not 39, as stated by them). We believe our sample size and number of positive GC specimens ($n = 45$) equates with a previous Australian-based study by some members of the ERG (15).

In our view, the diagnostic sensitivity, specificity, and predictive values were sufficient to pose a question regarding the need for confirmatory GC testing (10). We are not the first to pose this question. Similar results and conclusions from a comparable study in a low-prevalence population have been published (7). Importantly, we did not state in our publication that confirmatory testing of GC NAAT be abandoned in Australia or elsewhere.

While Lum et al. raise some valid points concerning the overall issue of GC NAAT in Australia, their criticisms and supporting evidence have not dealt specifically with the reported performance of the APTIMA GC NAAT assay in our population and dismissed the evidence from other overseas studies. This is unfortunate given the position of the authors; however, on the basis of the arguments they present here, we

see no justification for changing our view that the APTIMA GC NAAT assay does have a place in the laboratory diagnosis of *Neisseria gonorrhoeae* infection in our population.

REFERENCES

1. **Boel, C. H., C. M. van Herk, P. J. Berretty, G. H. Onland, and A. J. van den Brule.** 2005. Evaluation of conventional and real-time PCR assays using two targets for confirmation of results of the COBAS AMPLICOR *Chlamydia trachomatis*/*Neisseria gonorrhoeae* test for detection of *N. gonorrhoeae* in clinical samples. J. Clin. Microbiol. **43:**2231–2235.
2. **Boyadzhyan, B., T. Yashina, J. H. Yatabe, M. Patnaik, and C. S. Hill.** 2004. Comparison of the APTIMA CT and GC assays with the APTIMA combo 2 assay, the Abbott LCx assay, and direct fluorescent-antibody and culture assays for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. J. Clin. Microbiol. **42:**3089–3093.
3. **Bruisten, S. M., G. T. Noordhoek, A. J. van den Brule, B. Duim, C. H. Boel, K. El-Faouzi, R. du Maine, S. Mulder, D. Luijt, and J. Schirm.** 2004. Multicenter validation of the *cppB* gene as a PCR target for detection of *Neisseria gonorrhoeae*. J. Clin. Microbiol. **42:**4332–4334.
4. **Chernesky, M. A., and D. E. Jang.** 2006. APTIMA transcription-mediated amplification assays for Chlamydia trachomatis and Neisseria gonorrhoeae. Expert Rev. Mol. Diagn. **6:**519–525.
5. **Chernesky, M. A., D. H. Martin, E. W. Hook, D. Willis, J. Jordan, S. Wang, J. R. Lane, D. Fuller, and J. Schachter.** 2005. Ability of new APTIMA CT and APTIMA GC assays to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in male urine and urethral swabs. J. Clin. Microbiol. **43:**127–131.
6. **Gaydos, C. A., T. C. Quinn, D. Willis, A. Weissfeld, E. W. Hook, D. H. Martin, D. V. Ferrero, and J. Schachter.** 2003. Performance of the APTIMA Combo 2 assay for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in female urine and endocervical swab specimens. J. Clin. Microbiol. **41:**304–309.
7. **Golden, M. R., J. P. Hughes, L. E. Cles, K. Crouse, K. Gudgel, J. Hu, P. D. Swenson, W. E. Stamm, and H. H. Handsfield.** 2004. Positive predictive value of Gen-Probe APTIMA Combo 2 testing for Neisseria gonorrhoeae in a population of women with low prevalence of N. gonorrhoeae infection. Clin. Infect. Dis. **39:**1387–1390.
8. **Granato, P. A., and M. R. Franz.** 1989. Evaluation of a prototype DNA probe test for the noncultural diagnosis of gonorrhea. J. Clin. Microbiol. **27:**632–635.
9. **Johnson, R. E., W. J. Newhall, J. R. Papp, J. S. Knapp, C. M. Black, T. L. Gift, R. Steece, L. E. Markowitz, O. J. Devine, C. M. Walsh, S. Wang, D. C. Gunter, K. L. Irwin, S. DeLisle, and S. M. Berman.** 2002. Screening tests to detect Chlamydia trachomatis and Neisseria gonorrhoeae infections–2002. Morb. Mortal. Wkly. Rep. Recomm. Rep. **51:**1–38.
10. **Lowe, P., P. O'Loughlin, K. Evans, M. White, P. B. Bartley, and R. Vohra.** 2006. Comparison of the Gen-Probe APTIMA Combo 2 assay to the AMPLICOR CT/NG assay for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in urine samples from Australian men and women. J. Clin. Microbiol. **44:**2619–2621.
11. **Moncada, J., J. Schachter, E. W. Hook III, D. Ferrero, C. Gaydos, T. C. Quinn, D. Willis, A. Weissfeld, and D. H. Martin.** 2004. The effect of urine testing in evaluations of the sensitivity of the Gen-Probe Aptima Combo 2 assay on endocervical swabs for Chlamydia trachomatis and Neisseria gonorrhoeae: the infected patient standard reduces sensitivity of single site evaluation. Sex. Transm. Dis. **31:**273–277.
12. **National Pathology Accreditation Advisory Council.** 2000. Laboratory accreditation standards and guidelines for nucleic acid detection techniques, p. 19. Publications Production Unit, Australian Government Department of Health and Ageing, Canberra, Australia.
13. **National Pathology Accreditation Advisory Council.** 2006. Laboratory Accreditation standards and guidelines for nucleic acid detection techniques, p. 38. Publications Production Unit, Australian Government Department of Health and Ageing, Canberra, Australia.
14. **Smith, D. W., J. W. Tapsall, and G. Lum.** 2005. Guidelines for the use and interpretation of nucleic acid detection tests for Neisseria gonorrhoeae in Australia: a position paper on behalf of the Public Health Laboratory Network. Commun. Dis. Intell. **29:**358–365.
15. **Tabrizi, S. N., S. Chen, J. Tapsall, and S. M. Garland.** 2005. Evaluation of opa-based real-time PCR for detection of Neisseria gonorrhoeae. Sex. Transm. Dis. **32:**199–202.
16. **Tapsall, J. W., E. A. Limnios, N. L. Nguyen, I. Carter, G. Lum, K. Freeman, S. N. Tabrizi, S. M. Garland, D. M. Whiley, T. P. Sloots, and I. W. Chambers.** 2005. Cryptic-plasmid-free gonococci may contribute to failure of *cppB* gene-based assays to confirm results of BD ProbeTEC PCR for identification of *Neisseria gonorrhoeae*. J. Clin. Microbiol. **43:**2036–2037.
17. **Whiley, D. M., J. W. Tapsall, and T. P. Sloots.** 2006. Nucleic acid amplification testing for Neisseria gonorrhoeae: an ongoing challenge. J. Mol. Diagn. **8:**3–15.

Peter Lowe*
Peter O'Loughlin
Paul B. Bartley
Renu Vohra
Kathryn Evans
Michael White
Infectious Diseases and Immunology
QML Pathology
11 Riverview Place
Metroplex on Gateway, Murarrie 4172
Brisbane, Australia

*Phone: (07) 3121 4510
Fax: (07) 3121 4014
E-mail: peter.lowe@qml.com.au